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Identification of novel leads against Cystalysin of *Treponema denticola* to combat Periodontitis: A computational approach

Mahvish Khan[1], **Saif Khan**[2]✉, **Khurshid Ahmad**[3], **Mohtashim Lohani**[4], **Shafiul Haque**[5], **Hazza A Alhobeira**[6], **Ammar A Siddiqui**[7], **Shadab Mirza**[8], **Arshad Hussain**[9]

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Author Affiliation:
[1]Department of Biology, College of Science, University of Ha'il, Ha'il-2440, KSA
[2]Department of Basic Dental and Medical Sciences, College of Dentistry, University of Ha'il, Ha'il-2440, KSA
[3]Department of Medical Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk 38541, Republic of Korea
[4]Department of Emergency Medical Services, Faculty of Applied Medical Sciences, Jazan University, Jazan-45142, KSA
[5]Research and Scientific Studies, Unit, College of Nursing & Allied Health Sciences, Jazan University, Jazan-45142, KSA
[6]Department of Restorative Dentistry, College of Dentistry, University of Ha'il, Ha'il-2440, KSA
[7]Department of Preventive Dentistry, College of Dentistry, University of Ha'il, Ha'il-2440, KSA
[8]Department of Clinical Administration, College of Dentistry, University of Ha'il, Ha'il-2440, KSA
[9]Clinical Pharmacy Department, College of Pharmacy, University of Ha'il, Ha'il-2440, KSA

✉**Corresponding author**
Department of Basic Dental and Medical sciences, College of Dentistry, Hail University, Hail,
Saudi Arabia
Email: sf.khan@uoh.edu.sa / saifkhan.bio@gmail.com

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ABSTRACT

Present study attempts to determine efficient biogenic leads against proteolytic enzyme Cystalysin of *Treponema denticola*, a red complex pathogen, responsible for periodontitis. Cystalysin is known to play central role in the occurrence and progression of chronic generalized periodontitis. The method involves the preparation of library of compounds having biogenic origin from a large database of known compounds (ZINC). High throughput screening of 308035 compounds in the biogenic library was done to determine the top 100 best inhibitors depending on their binding efficiency. Top five Inhibitors were then subjected to exhaustive docking refinement to characterize the type and degree of interactions. Top inhibitors were found to undergo multiple hydrogen bonding and pi-anion interactions with active site residues critically important for the proteolytic activity of the enzyme. These inhibitors efficiently block the proteolysis due to Cystalysin and hence restrict the progression of periodontitis. Favorable ADMET properties of these ligands approve them to be used as potential drug like molecules against periodontal infections.

Keywords: Periodontitis, Cystalysin, Treponema, Drug discovery

1. INTRODUCTION

Periodontal disorders are a category of clinical conditions in which an inflammatory mechanism causes attachment apparatus damage, degradation of supporting alveolar bone, and, if left unaddressed, tooth decay (Guthmiller et al., 2002). According to the latest study by the Centers of Disease Control and Prevention in the United States, 47.2 percent of people aged 30 and over had periodontal disease. The periodontal disorder became more common as

people become older, with 70.1 percent of adults 65 and older having periodontal disease (Eke et al., 2012). Periodontitis is caused by an irregular host reaction to bacterial challenge as well as the deposition of dental plaque biofilm (Guthmiller et al., 2002).

Treponema denticola is a gram-negative, obligate anaerobic and extremely proteolytic bacterium, which is associated with human periodontal disease occurrence and progression. *T. denticola* releases Cystalysin, a 46-kDa, homodimeric pyridoxal 5′-phosphate (PLP) dependent,a hemolytic protein that lets the bacterium generate sulfide in the periodontal disease pocket by catalyzing the removal of L-cysteine (α,β-elimination) to give pyruvate, ammonia, and sulfide (Spyrakis et al., 2014; Chu et al., 1999; Khan et al., 2021). Sulfide, on the other hand, is responsible for hemolytic and hemoxidative processes, as well as gingival and periodontal tissue injury (Kurzban et al., 1999; Zhang et al., 2010). PLP was reported to be a Cystalysin cofactor (Chu et al., 1995). Similarly, like other PLP enzymes, it is capable of functioning in several modes, with extreme flexibility. It dissolves erythrocytes and converts hemoglobin to sulfhemoglobin and methemoglobin, which has also been recorded (Chu et al., 1999). PLP-binding residue (Lys 238) is an essential residue for the alpha-beta-elimination reaction catalyzed by Cystalysin, according to site-directed mutagenesis studies (Cellini et al., 2005). Consequently, Cystalysin can be considered a virulence factor and a possible target for the advancement of new medicines to combat periodontitis.

The high catalytic flexibility of Cystalysin has previously been demonstrated in detailed biochemical studies. Cystalysin catalyzes the racemization of all enantiomers of alanine, with a turnover number of seconds, and the half transamination of L and Dalanine, with such a turnover number of minutes, in addition to its physiologically significant protease activity (Cellini et al., 2005; Cellini et al., 2006). The use of computational methods is already identified as a possible alternative for discovering therapeutic molecules for a variety of lethal diseases quicker and cheaper (Alhobeira et al., 2021; Hassan et al., 2016). To produce potential therapeutic agents, natural ingredients are considered as the safest remedies. The ZINC database is a huge database of billions of compounds (Irwin et al., 2005). These can be categorized into subclasses based on molecular weight, nature of origin, neutral or charged, drug-like, sensitivity, lead-like, and fragments, among other factors.

The objective of structure-based/target-based virtual screening is to predict the best interactions between a large library of ligands and a molecular target to form a target-ligand complex (Maia et al., 2020). We employed state-of-the-art insilico screening of biogenic compounds against the catalytic site of Cystalysin to identify novel inhibitors with therapeutic potential.

2. MATERIALS AND METHODS

Library preparation

The ZINC database has been used to find natural compounds (https://zinc.docking.org) by selecting the 'biogenic' subset in the 'substances' category. A total of 308035 biogenic compounds were extracted, which included both primary metabolites (metabolites) and secondary metabolites (natural products). These compounds were downloaded in.sdf format and then imported into 'Discover Studio 2020' and proceed using the 'ligand preparation tool.'

Receptor Preparation

The 3D structures of the Cystalysin (PDB ID: 1C7N) were obtained by protein data bank (PDB) (https://www.rcsb.org/structure/1C7N, Krupka et al., 2000). The PDB file of the Cystalysin was cleaned by removing heteroatoms and water molecules and was prepared by the 'protein preparation wizard' of the DS2020 for further structure-based virtual screening purposes.

Structure-based virtual screening

The prepared library of biogenic compounds was screened against the catalytic site of 1C7N using the AutoDock Vina (version 1.1.2) program to for the identification of the potential lead compounds. The .sdf files of the biogenic compound library weretransformedto .pdbqt format by the Open Babel tool.

Pharmacokinetic properties calculation

Top-scoring molecules were further used to estimate drug-likeness, toxicity, and pharmacokinetic properties using the pkCSM web server (Pires et al., 2015). SMILE IDs of the molecules retrieved from the ZINC database were entered into the pkCSM tool to evaluate drug-likeness.

3. RESULTS

We present a computational screening of a library of biogenic compounds, comprising natural products and metabolites, obtained from the ZINC database against the active site of Cystalysin, a possible pharmacological target for periodontitis treatment. For virtual screening, the 3D structure of Cystalysin and a library of biogenic compounds were prepared. The inbound ligand marked the binding site (interaction site) skin of the 1C7N, and the X, Y, Z-axis was set to 15.6916, -1.9087, and 38.3366 for the subsequent screening steps. Table 1 shows the top 10 compounds based on the binding energy scores. In this study, we describe the top five compounds and their interactions with the active site of the 1C7N (Figure 1).

Table 1 Top 10 screened compounds

Compound ID	Binding Energy
ZINC000069482017	-9.1
ZINC000066112069	-8.9
ZINC000069481898	-8.7
ZINC000006017795	-8.5
ZINC000000006256	-8.4
ZINC000100406719	-8.4
ZINC000069482524	-8.4
ZINC000100406525	-8.1
ZINC000069482416	-8.1
ZINC000069481895	-8

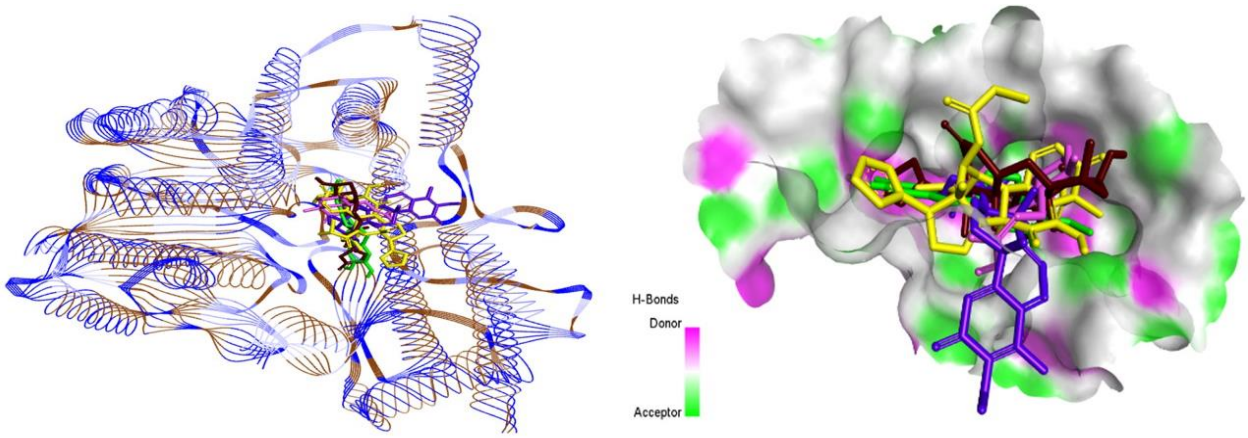

Figure 1 3D representation of Cystalysin interacting with top five screened compounds.

The top-scoring compounds showed almost similar patterns and with most of the active site amino acids (critical for proteolytic activity) of the 1C7N, namely Tyr123, Tyr124, Lys 238, Arg369, Arg369, Asp355, etc. the top 5 screened compounds are ZINC000069482017, ZINC000066112069, ZINC000069481898, ZINC000006017795, and ZINC000000006256, which showed strong binding with the active site residues of the receptor molecule (Figure 2). The ADMET parameters for all five compounds mentioned in this study were within an acceptable range (Table 2).

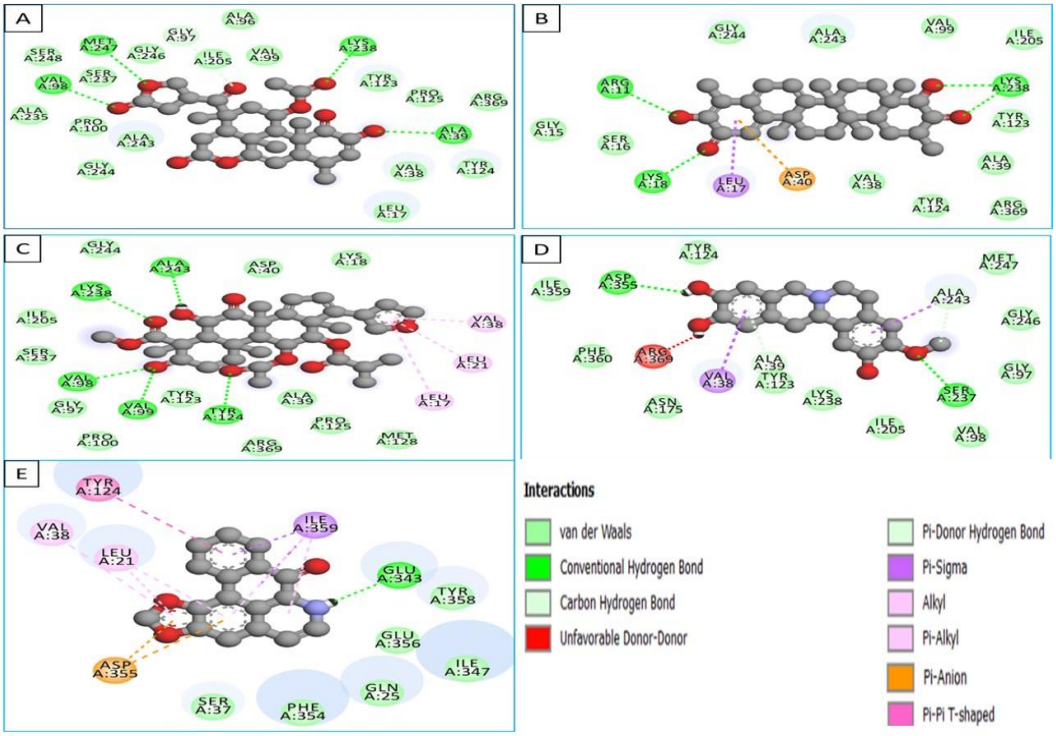

Figure 2 2D interaction of interacting residues of Cystalysin with A) ZINC000069482017, B)ZINC000066112069, C) ZINC000069481898, D) ZINC000006017795, and E)ZINC000000006256. Different types of interaction are displayed by different colors

Table 2 ADMET properties of top 5 lead compounds

Property	Model Name		Predicted Value				
			ZINC000069482017	ZINC000066112069	ZINC69481898	ZINC6017795	ZINC000000006256
Absorption	Water solubility		-4.144	-5.309	-4.848	-2.961	-4.777
	Permeability (Caco2)		0.851	1.067	0.642	0.958	1.326
	Intestinal absorption (human)		100	100	92.804	91.467	99.983
	Skin Permeability		-3.179	-3.199	-2.823	-2.735	-2.538
	P-glycoprotein substrate		Yes	Yes	Yes	Yes	Yes
	P-glycoprotein I inhibitor		Yes	Yes	Yes	No	No
	P-glycoprotein II inhibitor		No	Yes	Yes	No	No
Distribution	VDss (human)		0.131	-0.134	0.525	1.801	0.232
	Fraction unbound (human)		0.154	0	0.046	0.31	0.228
	BBB permeability		-1.013	0.485	-1.094	-0.7	0.235
	CNS permeability		-2.936	-1.26	-2.75	-2.296	-1.561
Metabolism	CYP2D6 substrate		No	No	No	No	No
	CYP3A4 substrate		Yes	Yes	Yes	No	Yes
	CYP1A2 inhibitior		No	No	No	Yes	Yes
	Inhibitor	CYP2C19	No	No	No	No	Yes
		CYP2C9	No	No	No	No	No
		CYP2D6	No	No	No	Yes	No
		CYP3A4	No	No	Yes	No	Yes
Excretion	Total Clearance		0.239	0.113	0.096	1.12	0.047
	Renal OCT2 substrate		No	No	No	No	No
Toxicity	AMES toxicity		No	No	No	No	Yes
	Max. tolerated dose (human)		-0.619	-0.449	-0.292	0.006	-0.43
	hERG I inhibitor		No	No	No	No	No

	hERG II inhibitor	No	No	No	Yes	No
	Oral Rat Acute Toxicity (LD50)	2.906	3.6	3.678	2.963	2.149
	Oral Rat Chronic Toxicity (LOAEL)	1.825	1.578	1.724	2.024	1.57
	Hepatotoxicity	No	No	No	No	No
	Skin Sensitisation	No	No	No	No	No
	T.Pyriformis toxicity	0.286	0.329	0.285	0.285	0.496
	Minnow toxicity	2.64	0	0.184	0.881	0.92

4. DISCUSSION

The periodontal infection has been extensively investigated as a disease through the years. Antimicrobial agents or topical antiseptics are delivered locally or systemically through this so-called antimicrobial method. However, these treatment methods may not be enough to stop or prevent periodontal tissue damage caused by the host (Bogdanovska et al., 2012). The first biogenic compound identified (ZINC000069482017) had the lowest binding energy (-9.1 Kcal/mol) and was found to interact with active site residues of 1C7N through various interactions such as hydrogen bonds (H bonds) and hydrophobic interactions. It was revealed that the receptor's Val98, Ala39, Met247, and Lys238 residues were interacting with this compound via an H bond (Figure 2A). Several other important residues (for example Tyr123, Tryr124, Val38, Ser248, Gly244 etc.) were also found in the interaction. The second compound identified (ZINC000066112069) was found to interact with active site residues of 1C7N through various interactions. It was observed that Lys238 formed two H bonds and Arg11 and Lys 18 formed one (Figure 2B). Several other crucial residues (such as Tyr123, Tryr124, Arg369, and so on) were also identified in the interaction. The pi-anion was formed by Asp40, and the pi-stigma bond was formed by Leu17 with the ligand molecule.

The third compound described (ZINC000069481898) was found to interact with active site residues of 1C7N through five H bonds (Val98,Val99, Tyr124, Lys238, and Ala243) and 3 hydrophobic interactions (Val38, Leu21and Leu17) and several by wander wall interactions (Figure 2C). The fourth and fifth compound (ZINC000006017795 and ZINC000000006256) were also found to interact with several active site residues of 1C7N. ZINC000006017795 was found to interact with two H bonds (Ser237 and Asp355) and Val38 showed pi-stigma interaction while several other residues viz., Tyr123, Tyr124, Lys238 etcformed wander wall interactions (Figure 2C). ZINC000000006256 formed H bond with Glu343 while Tyr124, Val38 Ile359 and Leu21 formed different various hydrophobic interactions including pi-stigma, alkyl, and pi-alkyl interactions. These compounds interacted with the receptor molecule, with the plurality of the residues involving the active site evidenced from the previous studies (Spyrakis et al., 2014).

Drug development is an expensive and time-consuming operation, with a high failure rate in clinical trials. Drug absorption, distribution, digestion, and excretion almost always ensured the general effectiveness and efficiency of clinical trial procedures (Hop et al., 2011). The pharmacodynamics of a proposed molecule that may be used as a drug include an understanding of ADME (Adsorption, Distribution, Metabolism, and Excretion).

5. CONCLUSIONS

The study successfully mines out natural products that may act as potential inhibitors of the periodontal drug target, Cystalysin, of the red complex pathogen, *Treponema denticola*. Acceptable ADMET properties successfully classify the discovered natural product biogenic molecules as efficient leads for the development safe and effective drugs for the better management of periodontitis.

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Conflict of Interest

The authors report no conflicts of interest.

Authors' contributions

This work was carried out in collaboration among all authors. 'Author Mahvish Khan and Saif Khan' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author Mohtashim Lohani, Hazza A Alhobeira, Ammar A Siddiqui, Shafiul Haque' and 'Mahvish Khan, Shadab Mirza and Shafiul Haque' managed the

analyses of the study. 'Saif Khan, Arshad Hussain and Mohtashim Lohani' managed the literature searches. All authors read and approved the final manuscript.

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Data and materials availability

All data associated with this study are present in the paper.

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